

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
22 January 2004 (22.01.2004)

PCT

(10) International Publication Number
WO 2004/007718 A3

(51) International Patent Classification⁷:
C07K 14/47, C12Q 1/68, A61K 48/00

C12N 15/11,

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(21) International Application Number:

PCT/EP2003/007516

(22) International Filing Date: 10 July 2003 (10.07.2003)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

02015532.1 10 July 2002 (10.07.2002) EP
02018906.4 23 August 2002 (23.08.2002) EP

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— *of inventorship (Rule 4.17(iv)) for US only*

Published:

— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
25 March 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: RNA-INTERFERENCE BY SINGLE-STRANDED RNA MOLECULES

(57) Abstract: The present invention relates to sequence and structural features of single-stranded (ss)RNA molecules required to mediate target-specific nucleic acid modifications by RNA-interference (RNAi), such as target mRNA degradation and/or DNA methylation.

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 03/07516

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/11 C07K14/47 C12Q1/68 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, MEDLINE, WPI Data, EMBASE, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	<p>TIJSTERMAN MARCEL ET AL: "RNA helicase MUT-14-dependent gene silencing triggered in C. elegans by short antisense RNAs" SCIENCE (WASHINGTON D C), vol. 295, no. 5555, 25 January 2002 (2002-01-25), pages 694-697, XP002257818 ISSN: 0036-8075 abstract page 694 - page 696 figure 1</p>	1-21
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☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- * & * document member of the same patent family

Date of the actual completion of the international search

30 October 2003

Date of mailing of the international search report

13. 01. 04

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/07516

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>BOUTLA A ET AL: "Short 5'-phosphorylated double-stranded RNAs induce RNA interference in drosophila"</p> <p>CURRENT BIOLOGY, CURRENT SCIENCE,, GB, vol. 11, no. 22, 13 November 2001 (2001-11-13), pages 1776-1780, XP002965213</p> <p>ISSN: 0960-9822</p> <p>table 1</p>	1-21
X	<p>ELBASHIR S M ET AL: "Analysis of gene function in somatic mammalian cells using small interfering RNAs"</p> <p>METHODS: A COMPANION TO METHODS IN ENZYMOLOGY, ACADEMIC PRESS INC., NEW YORK, NY, US, vol. 26, no. 2, February 2002 (2002-02), pages 199-213, XP002251055</p> <p>ISSN: 1046-2023</p> <p>figure 9</p>	1-21
X	<p>YU JENN-YAH ET AL: "RNA interference by expression of short-interfering RNAs and hairpin RNAs in mammalian cells"</p> <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 99, no. 9, 30 April 2002 (2002-04-30), pages 6047-6052, XP002259803</p> <p>April 30, 2002</p> <p>ISSN: 0027-8424</p> <p>abstract</p> <p>page 6049 - page 6050</p> <p>page 6047</p> <p>page 6052</p> <p>figures 2-4</p>	1-21
P,X	<p>MARTINEZ JAVIER ET AL: "Single-stranded antisense siRNAs guide target RNA cleavage in RNAi"</p> <p>CELL, vol. 110, no. 5, 6 September 2002 (2002-09-06), pages 563-574, XP002257819</p> <p>ISSN: 0092-8674</p> <p>page 569</p> <p>figure 7</p>	1-21
P,X	<p>SCHWARZ DIANNE S ET AL: "Evidence that siRNAs function as guides, not primers, in the Drosophila and human RNAi pathways"</p> <p>MOLECULAR CELL, vol. 10, no. 3, September 2002 (2002-09), pages 537-548, XP002257820</p> <p>ISSN: 1097-2765</p> <p>page 539 - page 541</p>	1-21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 03/07516

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claim 21 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.: 1-21(partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see PCT/ISA/210 annex

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-21(partially)

Present claims 1-21 relate to the use an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to RNA interference.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-21

Use of single-stranded RNA molecule having a length from 14 - 50 nucleotides wherein at least the 14 - 20 5 most nucleotides are substantially complementary to a target transcript for the manufacture an agent for inhibiting the expression of said target transcript.

2. claims: 22-29

Purified human RISC having a molecular weight of from up to about 150- 160 kDa.

3. claims: 31-32

An antisense siRNA precursor molecule in the form of a hairpin 25 stem-loop structure comprising 19 to 29 base pairs in stem, wherein at least 14 nucleotides in the stem are substantially complementary to a target transcript.
